Haematology-1

HAEMATOPOIESIS

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Aligned and Integrated Teaching: Anaemia module
Competencies addressed: PA 13.1, PY 2.4, 2.6, 2.7

- Learning Objectives:

➢To know the various organs of haematopoiesis during intra-uterine life and after birth.

➢To describe the stages in differentiation and maturation of blood cells in the marrow.

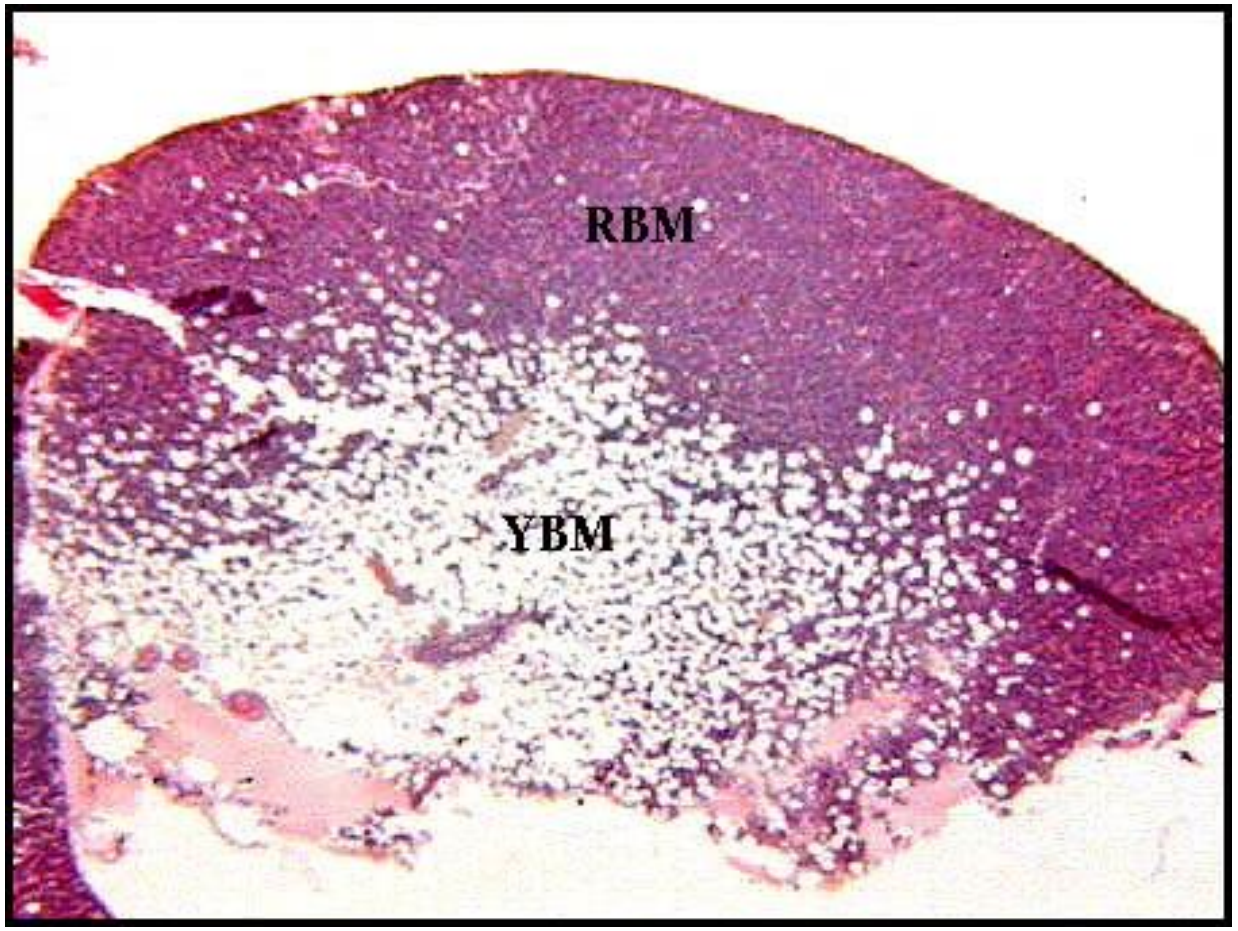
➢To discuss the role of haemopoietic growth factors in regulation of haematopoiesis.

➢To define extra medullary haematopoiesis

➢To enumerate causes and sites of extra medullary haematopoiesis

- **Haematopoiesis is defined as production of blood cells.**
- **Sites of haematopoiesis:**
- Blood cell progenitors first appear during third week of embryonic development in the **yolk sac.**
- Definitive **haematopoietic stem cells** (HSCs) arise several weeks later in the mesoderm of intraembryonic **AGM (aorta/ gonad/ mesonephros) region**.
- During third week of IUL, HSCs migrate to **liver** which is the chief organ of haematopoiesis till shortly before birth.
- HSCs also reside in **placenta** and this has important clinical implications.
- During fourth week of development HSCs relocate to **bone marrow**.
- At birth marrow throughout the skeleton is haematopoietically active and hepatic haematopoiesis dwindles to a trickle.
- After puberty, haematopoiesis ceases in distal bones and becomes restricted to **axial skeleton.**
- Thus, in a normal adult only half of the marrow space is haematopoietically active. This is called the red marrow. Rest is replaced by fat and is called the yellow marrow.

Red and yellow marrow

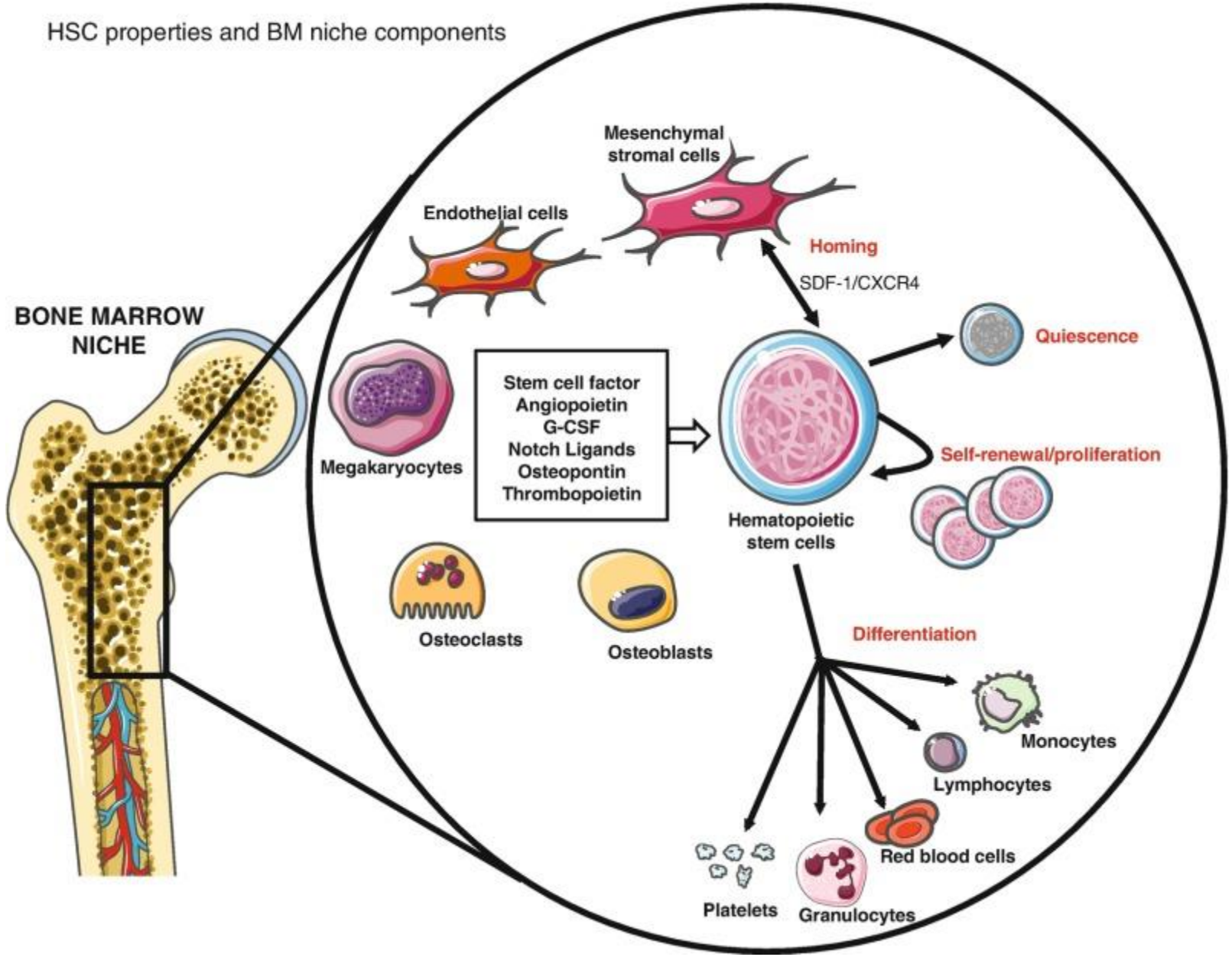


- **Haemopoietic Stem Cells:**
- HSCs have two essential properties:

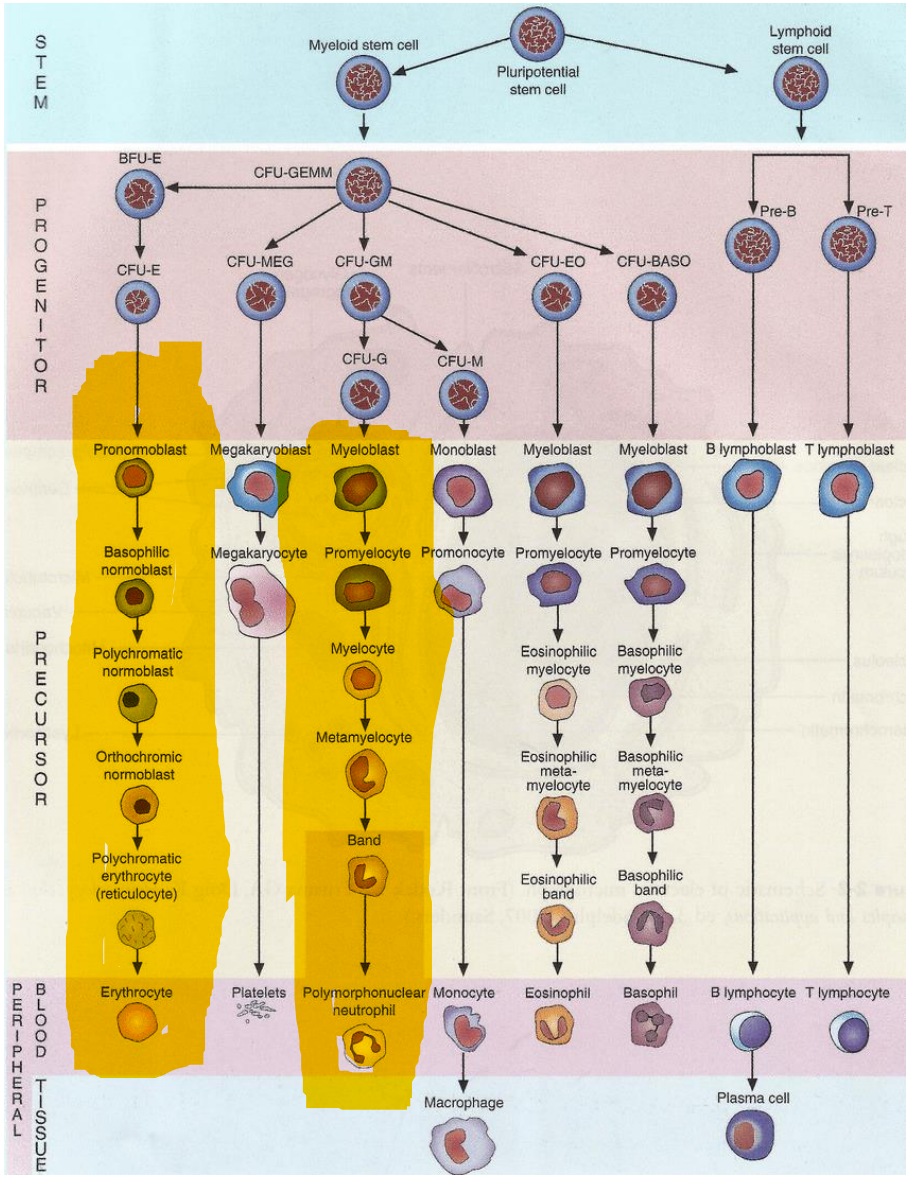
➢ **Pluripotency** i.e. ability of a single HSC to give rise to all the different types of mature blood cells.

➢ **Self renewal** i.e. when a HSC divides, at least one daughter cell must be a HSC and not a more differentiated or committed cell.

- HSCs can be mobilized from bone marrow into the peripheral blood under conditions of stress or in response to growth factors like **granulocyte colony stimulating factor (G-CSF).**
- HSCs give rise to **early multipotent progenitors**, having restricted differentiation potential i.e myeloid **(Myeloid Stem Cell**) or lymphoid **(Lymphoid Stem Cell)**.
- These early progenitors give rise to more **committed progenitors** called the **colony forming units (CFUs)** because they produce colonies of specific type of mature cells in culture.
- From the various committed progenitors are derived the **morphologically recognizable precursors** such as the **myeloblasts, proerythroblasts, megakaryoblasts** which are the immediate precursors of the mature granulocytes, red cells and platelets respectively.


HSC properties and BM niche components
BONE MARROW NICHE
Mesenchymal stromal cells
Endothelial cells
Homing
SDF-1/CXCR4
Quiescence
Megakaryocytes
Stem cell factor
Angiopoietin
G-CSF
Notch Ligands
Osteopontin
Thrombopoietin
Self-renewal/proliferation
Hematopoietic stem cells
Osteoclasts
Osteoblasts
Differentiation
Monocytes
Lymphocytes
Red blood cells
Platelets
Granulocytes

NORMAL HAEMATOPOIESIS



- **Haematopoietic Growth Factors:**
- Some growth factors such as **stem cell factor (also called KIT ligand) and FLT3 ligand** act through receptors expressed on **very early committed progenitors**.
- Others like **erythropoietin (EPO), granulocyte-macrophage colony stimulating factor (GM-CSF), granulocyte colony stimulating factor (G-CSF) and thrombopoietin (TPO)** act through receptors that are expressed only on **committed progenitors with more restricted differentiation potential.**
- These lineage specific growth factors regulate the production of blood cells by the marrow, via feedback control mechanisms and maintain their counts in the peripheral blood within appropriate ranges.
- Many diseases alter the production of blood cells. These include primary tumours of haematopoietic cells, certain genetic diseases, infections, toxins, nutritional deficiencies and chronic inflammation from any cause.
- The marrow responds to infectious or inflammatory challenges by increasing the production of granulocytes under the direction of specific growth factors and cytokines. Many other diseases result in defects in haematopoiesis leading to deficiencies of one or more type of blood cells.

Adult Reference Ranges for Peripheral Blood Cell Counts

Cell Type	Range
White cells (x $10^3/\mu L$)	4.8-10.8
Granulocytes (%)	40-70
Neutrophils (x $10^3/\mu L$)	1.4-6.5
Lymphocytes (x $10^3/\mu L$)	1.2-3.4
Monocytes (x $10^3/\mu L$)	0.1-0.6
Eosinophils (x $10^3/\mu L$)	0-0.5
Basophils (x $10^3/\mu L$)	0-0.2
Red Cells (x $10^3/\mu L$) Men Women	 4.3-5 3.5-5
Platelet (x $10^3/\mu L$)	150-450

HSCs and Haematopoietic Tumours

- Tumours of haematopoietic origin are often associated with mutations that block the maturation of progenitor cells or abrogate their dependence on growth factors.
- This results in unregulated clonal expansion of haematopoietic elements that replace normal marrow progenitors and also often spread to other haematopoietic tissues (spleen, liver and lymph nodes).
- In some cases the transformed HSCs retain the ability to differentiate along multiple lineages, while in others the transformed cell is a more differentiated progenitor with an abnormal capacity for self renewal.

IMPORTANT

Bone marrow microenvironment

- Bone marrow provides unique environment for proliferation and differentiation of the stem cells and precursor cells.
- Bone marrow is rich in stromal cells which support hematopoietic process. Stromal cell are capable of concentrating a lot of soluble factors(growth and differentiation factors) like erythropoietin, thrombopoietin, stem cell colony stimulating factors, IL-3 etc . IL-3 is responsible for stem cell proliferation.
- Stromal cells have cell to cell interaction with hematopoietic cells and directs their differentiation under the influence of appropriate growth and differentiation factors like erythropoietin, thrombopoietin, granulocyte monocyte colony stimulating factors and granulocyte colony stimulating factor.
- The bone marrow stromal cells express special surface adhesion molecules and stem cells have high affinity for these molecules. This accounts for the tendency of stem cells to home to the bone marrow.

Erythropoiesis

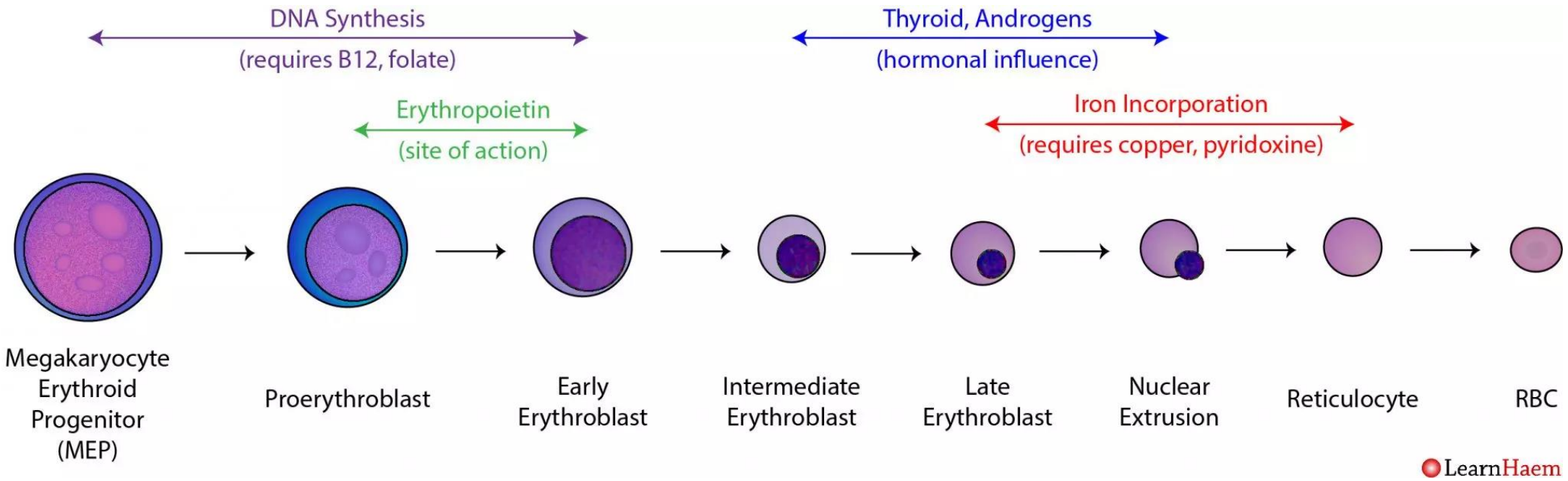
- The process by which common myeloid progenitor cells become fully mature red blood cells involves several stages. First, they become normoblasts (aka eryhthroblasts), which are normally present in the bone marrow only. Proerythroblast or pronormoblast is the first morphologically recognizable cell of the erythroid series.
- Secondly, they lose some organelles and their nucleus as they mature into reticulocytes, which can be thought of as immature red blood cells. Some of these are released into the peripheral circulation. Reticulocytes constitute 1-2% of the RBCs in the peripheral blood.
- Finally, reticulocytes lose their remaining organelles as they mature into erythrocytes, which are fully mature red blood cells. These normally survive for around 120 days.
- During this maturation process, there is nuclear extrusion – i.e. mature erythrocytes have no nucleus. Presence of nucleated red cells in the peripheral blood indicates the release of incompletely developed cells from the marrow. This can occur in pathology such as thalassaemia, severe anaemia or haematological malignancy.

Regulation of Erythropoiesis

- Erythropoiesis is driven mainly by the hormone **erythropoietin** (**EPO**), which is a glycoprotein cytokine.
- EPO is secreted by the kidney. It is constantly secreted at a low level, sufficient for the normal regulation of erythropoiesis. However, if the erythrocyte level becomes inadequate, the blood becomes relatively hypoxic. When there is a reduced partial pressure of oxygen (pO2) in the kidney, this is detected by the renal interstitial peritubular cells.
- In response, there is a surge in EPO production, which acts on the bone marrow to stimulate increased red blood cell production. This causes haemoglobin levels to increase, subsequently causing the pO2 to rise and therefore EPO levels to fall. The feedback loop is complete.
- **Clinical Relevance:**

➢ Chronic Kidney Disease: Chronic kidney disease often causes anaemia. In the damaged kidney, there is a reduced basal level EPO production and a reduced response to hypoxia leading to anaemia. To counteract this, patients can be given EPO injections as required.

➢ Drug Doping: However, exogenous EPO can also be used as a performance-enhancing drug among athletes. By stimulating increased red blood cell production, it increases the amount of haemoglobin available for oxygen-binding, thus improving the oxygen supply to muscles. However, studies have disagreed over whether this translates to an enhanced athletic performance.

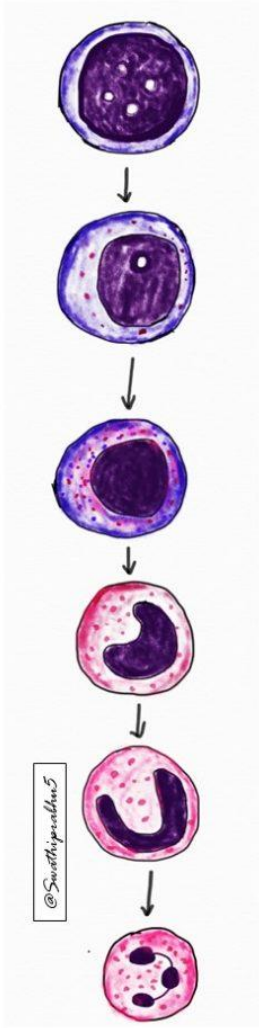


Erythropoiesis

Myelopoiesis or Granulopoiesis

- The process of formation of neutrophils, eosinophils and basophils from immature precursor cell is known as myelopoiesis.
- Myeloblast is the first morphologically recognizable cell of the granulocytic series.
- It is only after the appearance of the secondary or specific granules at the myelocyte stage, that a cell can be recognized as belonging to the neutrophil, eosinophil or basophil series. Cell division occurs up to this stage.
- Neutrophils once formed, remain in marrow for 5 or more days as a reserve pool. They have a life span of 1 to 2 days in circulation.

MYELOPOIESIS



Myeloblast: High nuclear to cytoplasmic ratio, immature chromatin, prominent 3 to 4 nucleoli

Promyelocyte: High nuclear to cytoplasmic ratio (but more cytoplasm than Myeloblast), round nucleus with immature chromatin, prominent nucleoli. Cytoplasm shows **primary azurophilic granules**

Myelocyte: Eccentric placed oval nucleus with no indentation, mature (condensed) chromatin, nucleoli are absent, more cytoplasm, less primary granules and more **secondary azurophilic granules**
The cells can undergo mitosis up to this stage

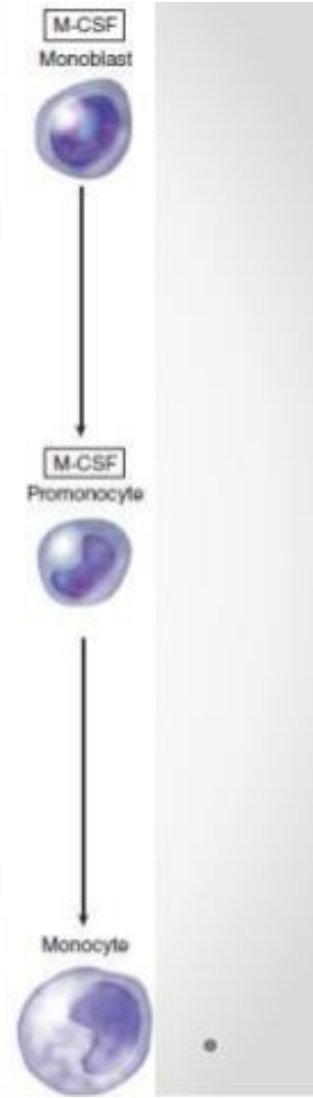
Metamyelocyte: Kidney bean-shaped nucleus with indented nucleus (indentation is less than half of the diameter of nucleus), mature chromatin, **only *secondary granules***

Band form (Juvenile): Kidney bean shaped with indented nucleus(more than half of the diameter of the nucleus), more cytoplasm, secondary granules

Segmented neutrophil: Mature nucleus divided in to 3 to 5 lobes connected by thin chromatin filament, secondary granules

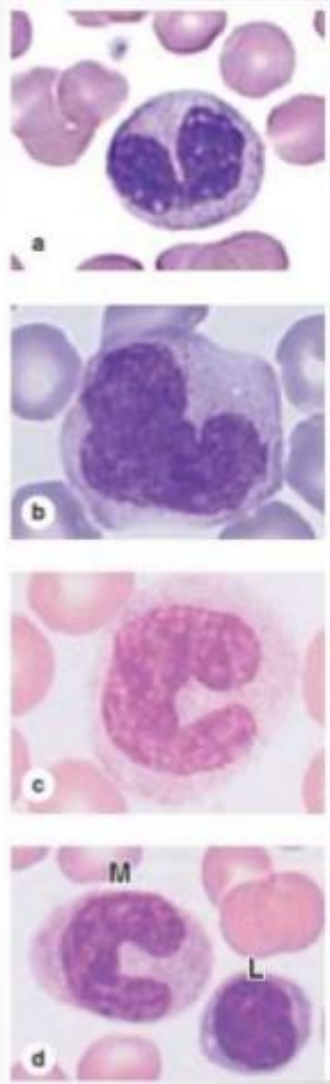
Monopoiesis

- The monoblast is a committed progenitor cell that is virtually **identical to the myeloblast morphologically**
- Further differentiation leads to the **promonocyte**, **a large cell** with **basophilic cytoplasm** and a **large, slightly indented nucleus**
- chromatin is **lacy** and nucleoli are **evident**.
- **Promonocytes divide twice** as they develop into monocytes.



Monocyte

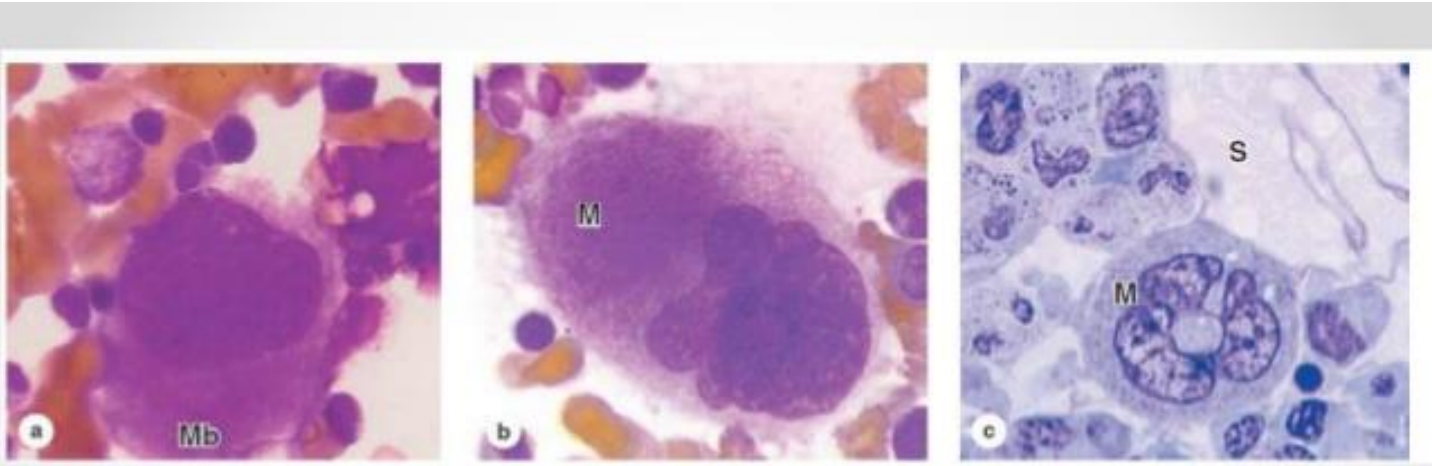
- **Monocytes** are large agranulocytes with diameters from **12 to 20 µm** that circulate as **precursors to macrophages** andother cells of the mononuclear phagocyte system.
- **Micrographs** of monocytes showing their **distinctive nuclei which are indented, kidney-shaped, or C-shaped.**
- Differentiating monocytes contain extensive **RER and large Golgi complexes** forming **lysosomes**, which are **observed as fine azurophilic granules at maturity**.
- **Monocytes circulate in blood for several hours and enter tissues where they mature as macrophages (or other phagocytic cells) and function for up to several months.**



Megakaryopoiesis

Megakaryoblast
↓
Promegakaryocyte
↓
Megakaryocyte
↓
Platelets

The process of platelet production is regulated by the hormone thrombopoietin



- (a) **Megakaryoblasts (Mb)** are very large, fairly rare cells in bone marrow, with very basophilic cytoplasm.
- (b) Megakaryoblasts undergo endomitosis (DNA replication without intervening cell divisions), becoming polyploid as they differentiate into **megakaryocytes (M)**. These cells are even larger but with cytoplasm that is less intensely basophilic.
- (c) Micrograph of sectioned bone marrow in which a megakaryocyte (M) is shown near sinusoids **(S)**.
- Megakaryocytes **produce all the characteristic components of platelets** (membrane vesicles, specific granules, marginal microtubule bundles, etc) and in a complex
- **process extend many long, branching pseudopodia-like projections** called **proplatelets**, from the ends of which platelets are pinched off almost fully formed.

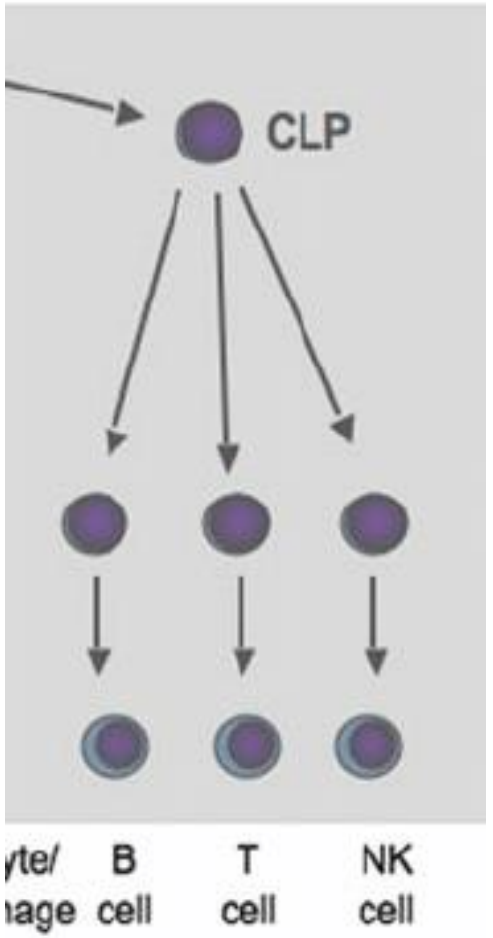
Lymphopoiesis—refers to the production of new lymphocytes, including B lymphocytes, T lymphocytes, and natural killer (NK) cells.

- B lymphocytes primarily produce immunoglobulins, also known as antibodies, and are key effectors of humoral immunity. They are distinguished by the presence of an immunoglobulin receptor complex, termed the B lymphocyte receptor. Plasma cells are terminally differentiated B lymphocytes that produce abundant immunoglobulin.
- T lymphocytes, effectors of cell-mediated immunity, possess T lymphocyte receptors that bind antigens prepared by antigen-presenting cells.
- A component of innate immunity, NK cells kill a variety of infected and tumor cells in the absence of prior exposure or priming.
- Main growth factors for B lymphocytes, T lymphocytes, and NK cells are IL-4, IL-2, and IL-15, respectively.
- Lymphocytes are derived from HSCs within the bone marrow. B lymphocyte development occurs in two phases, first in an antigen-independent phase in the bone marrow and ileal Peyer's patches (the site of B lymphocyte development in ruminants), then in an antigen-dependent phase in peripheral lymphoid tissues (such as spleen, lymph nodes, and mucosa-associated lymphoid tissue [MALT]). T lymphocyte progenitors migrate from the bone marrow to the thymus, where they undergo differentiation, selection, and maturation processes before migrating to the peripheral lymphoid tissue as effector cells.

Lymphopoiesis

The common lymphoid precursor (CLP) gives rise to the B cells, Tcells and NK cells.

The first identifiable progenitor of lymphoid cells is the **lymphoblast**.
A lymphoblast is a large cell capable of dividing 2 or 3 times to give rise to a lymphocyte.
As the lymphocytes develop, their nuclei become smaller, nucleoli become less visible and the cell size overall decreases.
In the bone marrow and the thymus these cells synthesize the specific cell surface proteins that characterize the B or T lymphocytes respectively.



Extramedullary Haematopoiesis

- Extramedullary hematopoiesis (EMH or sometimes EH) refers to hematopoiesis occurring outside of the medulla of the bone (bone marrow). It can be physiologic or pathologic.
- **Physiologic EMH** occurs during embryonic and fetal development; during this time the main sites of fetal hematopoiesis are liver and the spleen.
- **Pathologic EMH** can occur during adulthood when physiologic hematopoiesis can't work properly in the bone marrow and the hematopoietic stem cells (HSC) have to migrate to other tissues in order to continue with the formation of blood cellular components. In adults, the majority of hematopoiesis occurs in the bone marrow. Significant production in any other organ is usually the result of a pathological process. When red blood cell (RBC) numbers are low, the body induces a feedback mechanism aimed to increase the synthesis of RBCs, typically via the production of erythropoietin. If the loss of RBCs becomes severe, hematopoiesis will occur in the extramedullary spaces outside the bone.
- As the extra medullary sites lack the strict control of marrow microenvironment that allows only mature blood cells to be released into the circulation, extramedullary haematopoiesis is characterized by the presence of immature RBC precursors (nucleated RBCs/normoblasts) and granulocytic precursors (myelocytes and occasionally myeloblasts) in the peripheral blood. This is called **leucoerythroblastosis or leucoerythroblastic blood picture.**

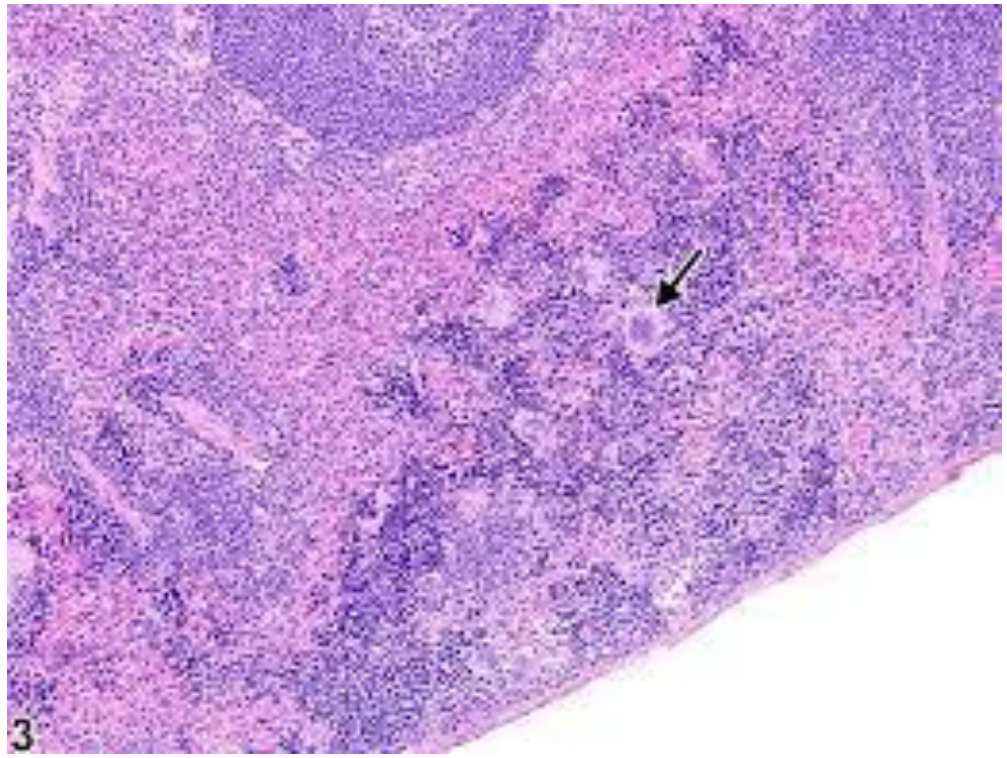
Causes of extramedullary haematopoiesis

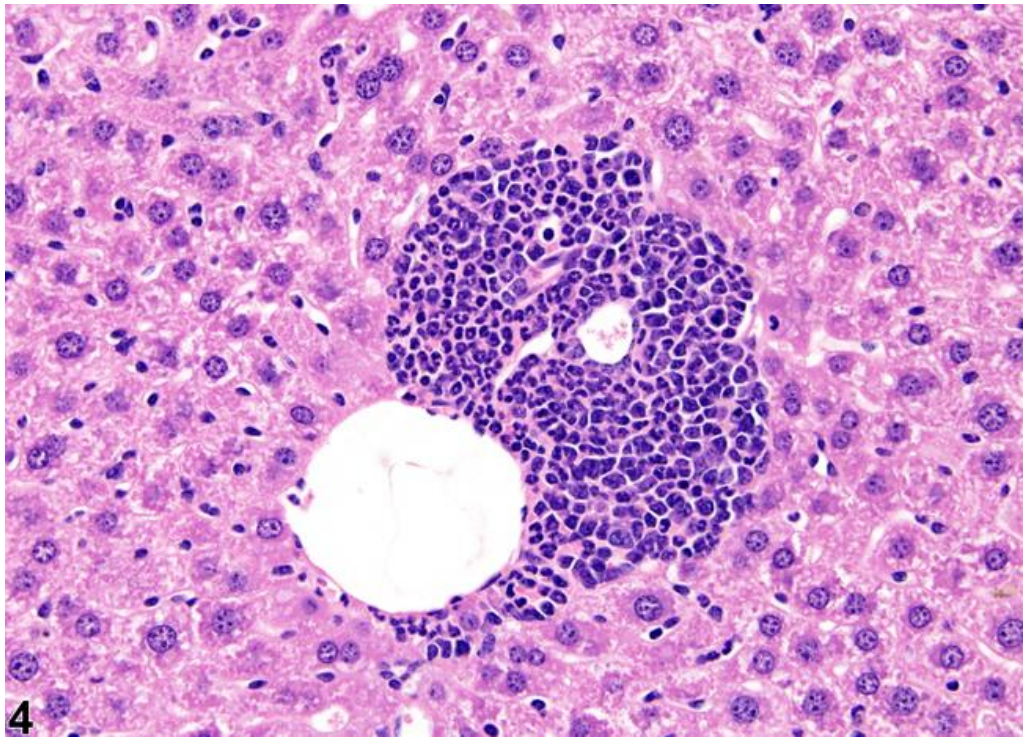
Reduced marrow production or peripheral destruction of blood cells	Displacement of marrow stem cells into peripheral circulation
Thalassemia	Primary myelofibrosis
Sickle cell disease	Osteopetrosis
Hereditary spherocytosis	Leukaemia
Autoimmune haemolytic anaemia	Lymphoma
Iron-deficiency anaemia	Granulomatous diseases
Megaloblastic anaemia	Metastasis
	Storage disorders

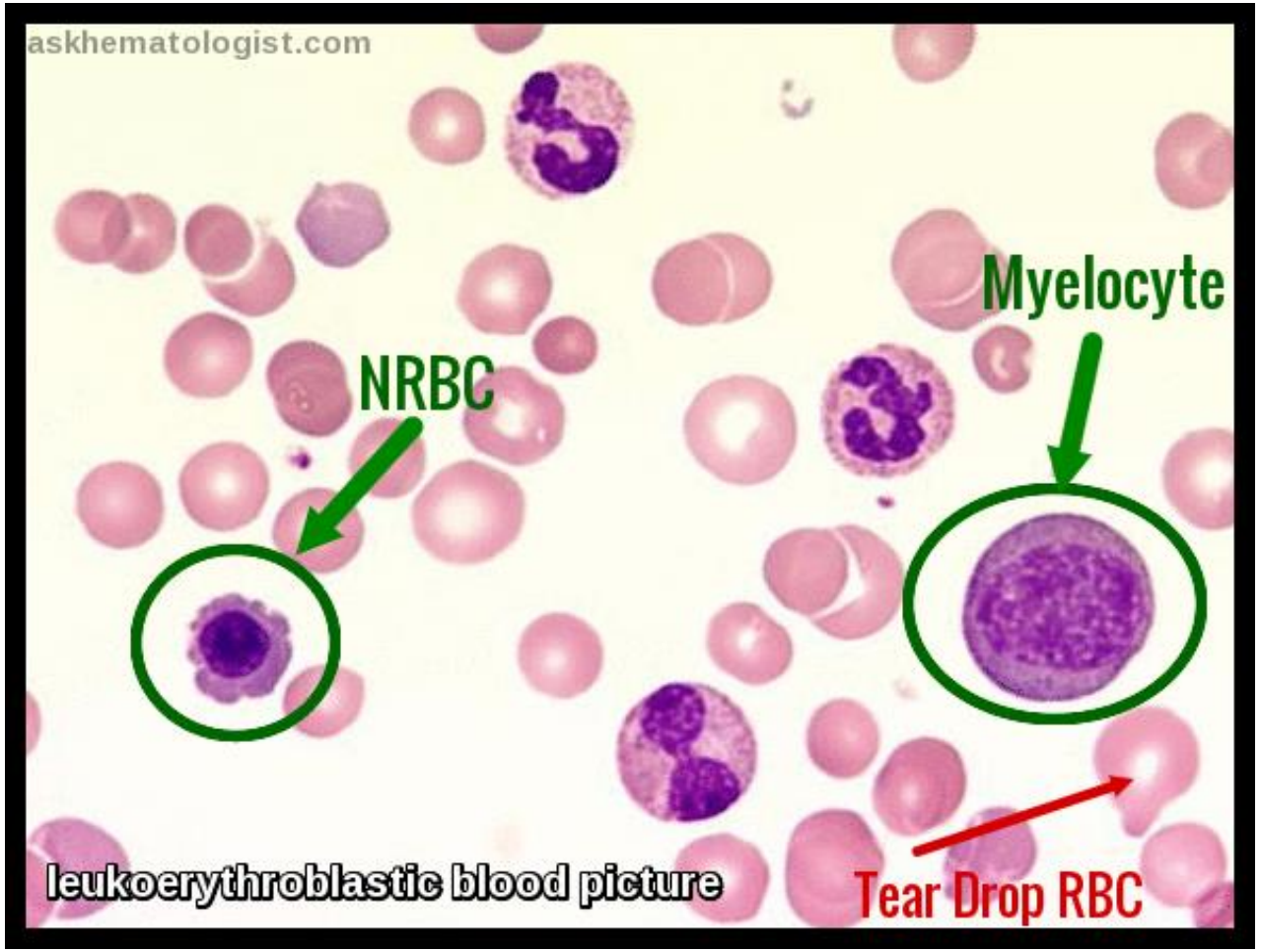
Sites of extramedullary haematopoiesis

- Sites of EMH can be widespread however, most common localizations are in the spleen, liver, and lymph nodes. Other manifestations occur in the thymus, heart, breast, prostate, broad ligaments, kidneys, adrenal glands, pleura, retroperitoneal tissue, skin, peripheral and cranial nerves, and the spinal canal.
- Spleen: During the postnatal period, the spleen becomes a frequent site of EMH whereas, during the embryonic stages of hematopoiesis, it is only a minor site. Despite the hypoxic/acidic conditions of the splenic microenvironment, supplied with a legion of macrophages making it inhospitable for HSCs, EMH usually occurs within the red pulp. Among the various organs associated with EMH, the spleen offers a unique site for evaluation of hematopoietic stem cell (HSC)/niche interactions.
- Liver: It is normal for infants to have hepatic EMH up until roughly 5 weeks of age. On the other hand, hepatic EMH in adults can indicate a pathological state. This includes transplantation, hepatic tumors, hepatic disorders, or sepsis. Hepatoblastoma, adenomas and hepatocellular carcinomas can also lead to EMH in adults. EMH is often observed within the hepatic sinusoids.
- Lymph nodes: EMH in the lymph nodes is usually associated with underlying hematopoietic neoplasms. Myeloproliferative neoplasms (MPNs) tend to result in EMH. If EMH is identified in the lymph nodes of an adult or infant, a hematologic evaluation, including blood cell counts, peripheral blood smear and potentially a bone marrow biopsy should be performed.
- Other sites: The following tissues may also be associated with EMH: thymus, heart, breast, prostate, fatty tissue, adrenal glands, kidney, periosteum, pleural cavity, para-vertebral regions, intra-spinal tissue, retroperitoneal tissue, skin, peripheral and cranial nerves, the spinal canal, pre-sacral region, nasopharyngeal region, para-nasal sinuses and numerous types of benign/malignant neoplasms. The most common sites of EMH associated with neoplastic disorders are the spleen, lymph nodes, skin, bone, small intestine, orbit, breast, cervix, nasal sinus, mediastinum and brain.

Extramedullary haematopoiesis in spleen (Left) and liver (Right)






askhematologist.com
NRBC
Myelocyte
leukoerythroblastic blood picture
Tear Drop RBC

Haematopoiesis concluded